# CHROMATOGRAPHIC STUDIES OF AMPHIPATHIC SUBSTANCES

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

**Master of Philosophy**

**IN**

**APPLIED CHEMISTRY**

By

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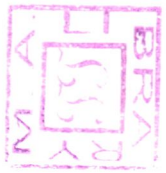
Under the supervision of

DR. ALI MOHAMMAD



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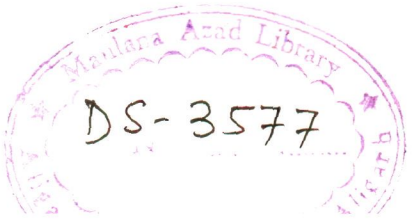
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# CERTIFICATE

*This is to certify that the work embodied in this thesis entitled,* ***"Chromatographic Studies of Amphipathic Substances"*** *is the original contribution of* ***Ms. Rubi Gupta,*** *carried out under my guidance and supervision, and is suitable for the award of degree of Master of Philosophy in Applied Chemistry from Aligarh Muslim University, Aligarh.*

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***Rubi Gupta***

*Dedicated*

*to*

*My Beloved Parents*

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# Chapter-1

# General Introduction

# CHROMATOGRAPHY

In spite of the popular belief and general acceptances of the contribution of *Tswett* as being the real discoverer of chromatography (literally "colour writing" from the Greek), the starting of chromatography predated to the work of *F.F. Runge* who investigated the separation of coloured substances (i.e. dyes) on paper (1). Chromatography was defined by *Cassidy* (2) as "a separation process applicable to essentially molecular mixtures". The earlier work carried out by *Goppelsroeder* (3) and *Schonbein* (4) on chromatography separation of substances of filter paper had been included in a report published by *Fischer* and *Schmidner* (5) in 1892. However, the concept of separation on columns may be attributed to *Reed's* work, which was followed by *Day* who separated petroleum fractions with the help of columns (6, 7) the paper published in 1906 by *M. Tswett,* a lecturer of Botany at the University of Warsaw provided the first description in nearly modern terms of chromatographic separation (8). He realized the resolution of different components of pigments as coloured bands (like spectrum of light rays) on a calcium carbonate column and he termed it as "*chromatogram*". The actual importance of *Tswett* work remained dormant until about 1931, when separation of plant carotene pigments were reported by prominent organic chemist *Kuhn* (9,10). In 1941,

*Martin* and *Synge* (11,12) laid another milestone in development of chromatography by reporting their discovery of liquid-liquid partition chromatography.

Chromatography is a physical methods of separation in which the components to be separated are distributed between two phases, namely (i) stationary phase, which can be a solid or a liquid support on a solid (ii) mobile phase (either a gas or a liquid) which flows continuously through the stationary phase. The separation of individual components results primarily due to differences in their affinity for the stationary and mobile phase.

## CLASSIFICATION OF CHROMATOGRAPHY

The chromatography system can be classified according to (a) state of aggregation of the phases, (b) physical arrangement of the phase and (c) mechanism operating in the distribution equilibrium.

According to the mode of separation of mechanism, chromatography can be adsorption, partition, ion exchange, size exclusion, electro-chromatography etc. A simple classification chromatographic methods is summarized in Table 1.1 below:

**Table 1.1: Classification of chromatographic methods**

| Types of Chromatography | Examples |
|---|---|
| Adsorption chromatography | Column chromatography, thin layer chromatography, gas-solid chromatography |
| Partition chromatography | Paper chromatography, reversed-phase thin layer chromatography, classical liquid-liquid chromatography |
| Modified partition (or bonded phase chromatography) | High pressure liquid chromatography (HPLC) and higher performance thin-layer chromatography (HPTLC) |
| Ion-exchange chromatography | Cation and anion exchange chromatography |
| Exclusion chromatography | Ion-exclusion and gel permeation chromatography, molecule or sieve chromatography |
| Electrochromatography | Capillary and zone electrophoresis |

Since the work presented in this thesis is mainly based on the use of thin layer chromatography as an analytical tool, it is necessary to mention the salient features of this technique. The following

paragraphs are devoted to cover all important aspects of the development and current state of art procedure of thin layer chromatography as used for the analysis or organic and inorganic substances.

## THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC), a subdivision of liquid planar chromatography in which the mobile phase a (liquid) migrate through the stationary phase (thin layer of porous sorbent on a flat inert surface) by capillary action. It is rapid, simple, versatile, reasonably sensitive and in expensive analytical tool, which is applicable for both quantitative and qualitative analysis of several compounds.

The beginning of TLC may be attributed to *Beyerink* (13) who reported the separation of suphuric acid and hydrochloric acid in the form of fine rings of thin layer of gelatin using a visualizing agent. Following the same methods *Wijsman* (14) identified the presence of two enzymes in malt *diastase* using a fluorescent method for detecting separated enzymes on thin layer. In 1938, *N.A. Izmailov* and *M.S. Schraiber*, separated certain medicinal compounds on binder free horizontal thin-layer of alumina spread over a glass plate (15). Since 1958, when Stahl introduced the term "*thin layer chromatography*"

and standardized procedures, materials and nomenclature (16,17), the effectiveness of this techniques for separation was realized.

A major breakthough in the field of TLC came in the early 1960's with the availability of precoated plates. It had recently been realized that modern high performance thin layer chromatography (HPTLC) initiated in 1975, rivals high pressure liquid chromatography (HPLC) and gas chromatography in its ability to resolve complex mixtures and to provide faster quantification.

## TLC PROCEDURE

TLC is an off-line process in which various steps as illustrated in Figure. 1.1 are carried out independently.

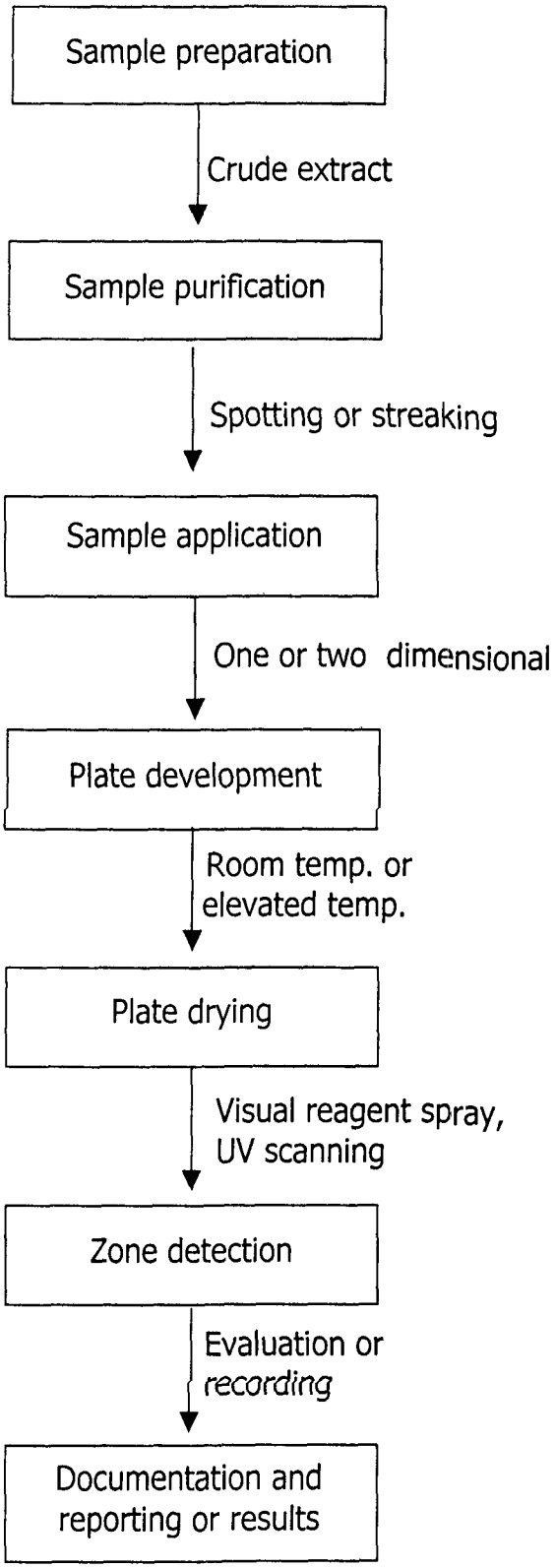



**Figure 1.1: Schematic diagram showing the steps involved in a TLC process**

## SOIL

The term soil is derived from the Latin word '*Solum*' which means floor. It is a complex heterogeneous medium consisting of minerals, organic solids and aqueous as well as gaseous components. The minerals present are usually rock fragment and secondary minerals (Phyllo- silicates or clay minerals), oxides of Fe, Al and Mn and sometimes carbonates usually $CaCO_3$. The term 'oxides includes' all forms of oxides including hydrous oxides and oxyhydrooxides.

Composition of soils: In an ideal soil surface the following components observed (a) Mineral matter-40% (b) Organic matter-10% (c) Soil matter-25% (d) Soil air-25%.

### (a) Mineral Matter

Mineral particles are formed from disintegration of rock by physical and chemical weathering processes. The size and grouping of the various particles affect the characteristics of soil such as texture. Primary minerals come directly from rock such as quartz, feldspars and micas. Secondary minerals such as clays and salts are formed primary particles by weathering. The smallest particle size constituents (<0.002mm) is clay, which is the most active and reactive portion of the soil (18). Some important clay minerals in relation to soil are (i) Kaolinite (ii) Montmorillonite (iii) Illite

(iv) Chlorite (v) Amorphous clays. The clay of first three types are strongly adsorbs organic cations (19-21).

**(b) Soil Organic Matter (SOM)**

The organic matter content of soil is generally found in smaller quantities that is 1-5% by weight in a top soil and decreases with depth. Organic matter and clay minerals are the two important component of the soil which are responsible for ion exchange property (22-23). Soil organic matter have non humic and humic substances. The non humic substances comprises unaltered biochemicals such as amino acid carbohydrates organic acids, fats waxes. Humic substances are series of acidic yellow to black colour poly electrolytes of moderately high molecular weight. They have wide variety of functional groups including carboxyl, phenolic hydroxyl carbonyl, ester and possibly quione and methoxy groups (24,25).

**(c) Soil Water**

In soil, water is supplied to plants through the roots. It "lubricates" the soil allowing root penetration which is necessary for microbial activity. The soil water contents varies with soil texture and ranges from 5.1 to 11.9%

**(d) Soil Air**

Percentage composition of atmospheric air (by volume) may be approximately taken as $N_2$ = 78.8, $O_2$=20.97, $CO_2$ = 0.03. Composition of $CO_2$ which increases after rainfall, probably because of increased nitrification and decomposition of organic matter.

## PHYSICO CHEMICAL PROPERTIES OF SOILS

It includes the distribution of mechanical composition such as (sand, silt and clay), soil pH, electrical conductivity, cation exchange capacity, exchangeable cations and available nitrogen.

**(a) Determination of Mechanical Composition of Soil**

The mechanical composition of soil sample was determined by International Pipette method, in which 10gm of the surface soil, previously passed through a, 7 mesh (B.S.S.) sieve was dispersed in water after treating with 30% $H_2O_2$ and 0.2 HCI using 50ml sodium oxalate (8g/l) as dispersing agent. The percentage of sand was calculated from the weight of the residues left behind on 200 mesh (B.S.S) sieve. The suspension was diluted to 500ml and transferred to a graduated boiling tube, which was immersed in a constant temperature water bath at $25^{\circ} \pm 5^{\circ}C$ throughout the course of pipetting. A 10ml sample was pipetted out carefully at specified intervals of time from a depth of 10cm, dried and weighed. The

percentage of clay was then calculated from the weight of residues. The percentage of silt was calculated by substracting the sum of percentage of all the fractions (Sand plus clay) from 100.

**(b) Determination of pH**

The pH of the soil sample was recorded with Elico pH meter. A soil mixed with water in 1:5 ratio was used for measuring the pH of the soil sample.

**(c) Determination of Electrical Conductivity (EC)**

The electrical conductivity of the soil sample (Soil + Water, 1:5) was measured at 30 $\pm$ 5°C using a conductometer (Elico, India).

**(d) Determination of Organic Matter**

The organic matter of the soil sample was estimated by using method of *Walkely* and *Black*. Soil sample (2g) was taken in 500ml conical flask, 10ml of 1.0N potassium dichromate solution and 20ml of concentrated sulphuric acid were added to flask. The flask was shaken vigorously several times and allowed to stand for 30 minutes. Thereafter, 200ml of distilled water, 10ml of orthophosphoric acid and 1ml of diphenylamine indicator were added to flasks. The excess of unreacted potassium dichromate was titrated against standard 0.5N ferrous ammonium sulphate solution till the violet colour change to

green. From the volume of ferrous ammonium sulphate solution used, the organic carbon was calculated by using the expression:

$$\text{Organic carbon (\%)} = \frac{(\text{Blank titre} - \text{Actual titre}) \times 0.003 \times N \times 100}{\text{Weight of dry soil in g}}$$

Where,

N is the concentration of ferrous ammonium sulphate.

The value of organic carbon was converted to organic matter by multiplying with the factor 1.724.

**(e) Determination of Cation Exchange Capacity (CEC)**

A 5g soil sample was taken in a 100ml conical flask and the soluble salts were washed out by treating the soil with 5ml of 0.05N HCl and finally with distilled water. It was further treated with sodium acetate (pH 5) for 30 minutes with intermittent stirring. The treated sample was given five times washing with standard $CaCl_2$ solution. The excess salt was removed by washing with aqueous acetone solution ($H_2O$+acetone, 20+80) until the excess $CaCl_2$ is removed, as indicated by a negative $AgNO_3$ test for chloride ion in the final washing. The calcium ions were exchanged from Ca saturated soils by means of treating it with a neutral sodium acetate solution. The washing were collected and utilized in the determination of exchanged $Ca^{2+}$ by titrating with a standard EDTA solution, using 10ml of buffer solution ($NH_4Cl$- $NH_4OH$) of pH 10

and eriochrome black "T" indicator in the presence of 1ml of 2% NaCN soln. as masking agent for interfering ions. A reagent blank was also run simultaneously to avoid any error due to impurities. The blank reading was subtracted from the reading of calcium determination. From the volume of EDTA solution used, the value of cation exchange capacity was calculated by using the following expression:

$$\text{Cation exchange capacity (meq.100}^{-}\text{g soil)} = \frac{V \times N \times 100}{\text{Weight of soil in g}}$$

**(f) Determination of Exchangeable Cations**

50g dried soil sample was taken into 250ml conical flask and 100ml of 1.0N $NH_4OAC$ was added. The contents of the flask were shaken for 20 minutes and allowed to stand over night. The soil contents were then transferred into a buckner funnel, in which a moist Whatman filter paper No. 42 had been seated by a gentle suction. The soil sample were leached with an additional 400ml of $NH_4OAC$. The filterate containing $NH_4OAC$ extract of the soil was evaporated to dryness on a steam plate, the dark coloured residue containing organic matter was treated with 2ml of 30% $H_2O_2$ and 2ml of 6N $HNO_3$. the contents were treated to dryness on a steam plate. The dried organic matter free residue was then dissolved in 10ml of 6N HCl and diluted with distilled water. The volume of the filtrate obtained after

filtration of contents through Whatman filter paper No. 42 was made to 100ml. This solution was used for the determination of exchangeable cations (e.g. $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$ and $K^{+}$) in soil sample. Exchangeable calcium plus magnesium was estimated in the 10ml of above solution by EDTA titration, using a half test tube of buffer solution (pH-10) and 4-5 drops of eriochrome black "T" indicator. Calcium was also estimated separately using mureoxide indicator with 10% KOH as recommended by *Jackson* (26). The volume of EDTA solution for magnesium was calculated by subtracting the volume for calcium from the volume of calcium plus magnesium used. The exchangeable sodium and potassium as estimated in the above solution using "Systronics" flame photometer. Ion exchange refers to the exchange between the counter- ions balancing the surface charge on the colloids and the ions in the soil solution (27). It has the following characteristic reversiable diffusion controlled & stoichiometric process which involved the preference for one ion over another by the adsorbent (28).

**(g) Determination of Available Nitrogen**

Available nitrogen was determined by the method of Hesse (29).

(i) Determination of ammonium nitrogen: A 5g soil sample was taken in 100 ml glass stopper conical flask. A 50ml of 2M KCL solution was added in it. The flask was shaken vigoriously for an hour and the

soluble contents were extracted through Whatman filter paper No. 42. A 10 ml solution from extracts was taken in distillation flask and diluted with 50 ml distilled water. A 0.5g magnesium oxide was added through long-stemmed funnel into the flask. Ammonia was distilled into a solution of 5ml of boric acid containing mixed indicator through a condenser until the final volume of the distillate was reached to about 30 ml. The boric acid was then titrated with M/70 HCl by using a micro burette until the green colour changed to pink. A blank was also run in the same way. The exchangeable ammonium nitrogen was calculated using the relation:

1 ml of M/70 HCl = 0.2 mg of nitrogen.

(ii) Determination of nitrite nitrogen plus nitrate nitrogen: After distillation of ammonia nitrogen, the stopper of the distillation flask was removed and a 0.2g of Devarda alloy and 50ml of distilled water were added to it. The distillation flask was stoppered and then ammonia was distilled in a fresh portion of 5ml boric acid solution until 30ml distillate was collected and then it was titrated with M/70 HCl. The value of nitrite nitrogen plus nitrate nitrogen were calculated in the same manner as described in the determination of ammonium nitrogen.

(iii) Determination of nitrate nitrogen: After distillation of ammonia nitrogen the stopper of the distillation flask was removed and 1 ml of

2% aqueous solution of sulphamic acid was added to the flask. The distillation flask was swirled for few seconds to destroy $NO_2$. And then 0.2g Devarda allog and 50ml distilled water were added in distillation flask. It was stoppered and ammonia was distilled in a fresh portion of boric acid solution. About 30ml distillate was collected in each case and tirated with M/70 HCl. The value of nitrate nitrogen was calculated in same manner as described in the determination of ammonium nitrogen.

(iv) Determination of Nitrite Nitrogen:

Nitrite nitrogen = (Nitrilte nitrogen+Nitrate nitrogen)–nitrate nitrogen

## SOIL THIN LAYER CHROMATOGRAPHY

Soil thin layer chromatography was successfully utilized in 1968 by *Helling* and *Turner* for the detection of pesticides movements using different types of soil as static phase. The movement of substances or their separations in TLC can be affected by altering the conditions of the static phase (soil) of diverse nature, developer and applied substances. Thus soil TLC provides a very fascinating field of research which can be utilized for investigating problems in various applied and non applied fields. Investigation (30) regarding amino acids metabolism in soil and plants uptake of amino acids in the form of nitrate by plants from soil and amino acids migration in soil by TLC. Thin layers of soil are prepared by spreading on uniform film

(0.15- 2.00 mm) of soil slurry over a glass plate with the help of application and allowing it to dry at room temperature. Thin-layer plates are developed to a distance of 10 cm starting from the point spotted samples. After the development the plates are removed from jars, dried and the samples are detected by spraying suitable chromogenic reagents as detector.

The movement of the compound through the soil layer is measured in terms of $R_F$ values which can be calculated as

$$R_F = \frac{\text{Distance traveled by solute from the point of application}}{\text{Distance travelled by solvent from line of sample application}}$$

The $R_F$ value called retardation factor, depends upon the nature of sorbent, layer thickness, room temperature, sample volume, relative humidity and mode of development technique.

**AMPHIPATHIC SUBSTANCES**

Those substances owing to the presence of both non-polar (hydrophobic) (Lipophilic) and polar (hyprophilic) lipophobic groups, these substances reffered to as amphipathic (tensio active, heteropolar or polar-non-polar substances. These substances has one polar end and other non-polar end each end of these substances to display its own solubility behaviour. Those substances which possess these two groups are surfactant, lipids and some amino acids.

## CHROMATOGRAPHIC SYSTEM

Chromatographic systems includes as (a) stationary phase (b) Mobile phase.

### (a) Stationary Phase (Adosrbent)

A large number of sorbents are available which can be used in TLC. However, more commonly used sorbents are silica gel, alumina, cellulose and kieselguhr and soil.

### (b) Mobile Phase (Solvent system)

With a particular sorbent layer, the separation possibility of a complex mixture is greatly improved by the proper selection of mobile phase. The following mobile phases have been used as developers:

*(i)* ***Inorganic Solvent Systems:*** Solutions of mineral acids, bases, salts and mixtures of acids, bases or their salts.

*(ii)* ***Organic Solvent Systems:*** Acids, bases, hydrocarbons, alcohols, amines, ketones, aldehydes and ester their mixtures in different proportions.

*(iii)* ***Mixed Aqueous Organic Solvent Systems:*** Above-mentioned organic solvents mixed with water, mineral acids, inorganic bases or dimethylsulphoxide and buffered salt solutions.

*(iv)* ***Surfactant-Containing Solvent Systems:*** Solutions of surfactants (SDS, CTAB, CPC, Tx-100) in various concentrations.

**Surfactant- Mediated Systems**

Since work presented in this thesis is related to the use of surfactants mediated mobile phase systems, it is worthwhile to give a brief idea about their behaviour om aqueous medium. The following paragraphs are devoted to highlight their utility as eluent.

These system contain surfactant as one of the components of the mobile phase. Surfactant in the aqueous mobile phase can be used in the following ways:

(a) As monomer surfactants where the concentration of surfactant in aqueous mobile phase is restricted to well below the critical micelle concentration (CMC) of the surfactant. These mobile phases are most suited to separate ionic species by ion-pair chromatography (IPC).

(b) As surfactant micelles where the surfactant concentration is kept well above its CMC value. In such cases, the mobile phase is composed of surfactant molecules in the form of monomers and aggregates (or micelles). These mobile phases are very useful for simultaneous separation of ionic and non-ionic compounds by micellar liquid chromatography (MLC).

(c) As microemulsion where surfactant in the presence of water, as oil (hydrocarbon) and co-surfactant (i.e. medium chain length amine or alcohol) is used transparent solution.

Surfactants are long chain amphiphilic organic or organometallic molecules containing a highly polar (hydrophilic or lipophobic) or "ionic head group" attached to a non-polar (hydrophobic or lipophilic) hydrocarbon tail of varying chain length. The "head group" is cationic (e.g. ammonium or pyridinium ion), amionic (e.g. hydroxy compounds) or zwitterionic (e.g. amine oxide, carboxylate or sulphonate betain) and the hydrocarbon tail which may contain at least eight carbon atoms. Depending upon the nature of hydrophilic groups, surfactant can be classified as anionic [$R\text{-}X^- M^+$], cationic [$R\text{–}N^+ (CH_3)_3 X^-$], zwitterionic [$R\text{-} (CH_3)_2 N^+ CH_2 X^-$] and non ionic [$R (CH_2CH_2)_m OH$], where R is a long aliphatic hydrocarbon chain, $M^+$ is a metal ion, $X^-$ is a halogen, $COO^-$ or $SO_4^{2-}$ and m is an integer.

**Micelles**

Surfactant molecules comprising of hydrophobic moieties tend to exhibit a considerable degree of self-organization when dissolved in aqueous solution. Above a certain concentration level termed as critical micelles concentration (CMC), the surfactant molecules in solution (water or organic solvents aggregate to form micelles. The

process of micelle formation is called "micellization" There is a very small concentration range below which aggregation to micelles is absent and above which association leads to micelle formation. This narrow concentration range during which micelle formation occurs is called the CMC. A 25°C and 1 atmospheric pressure, the CMC is typically less than 20mm, with each micelle consisting of 40-140 monomers. A conventional model of micelles is that proposed by *Hartly*. Figure. 1.2 which is very useful for visualization of a micelle. The various structures formed in aqueous solution on increasing the concentration of surfactant are illustrated in Figure 1.3.

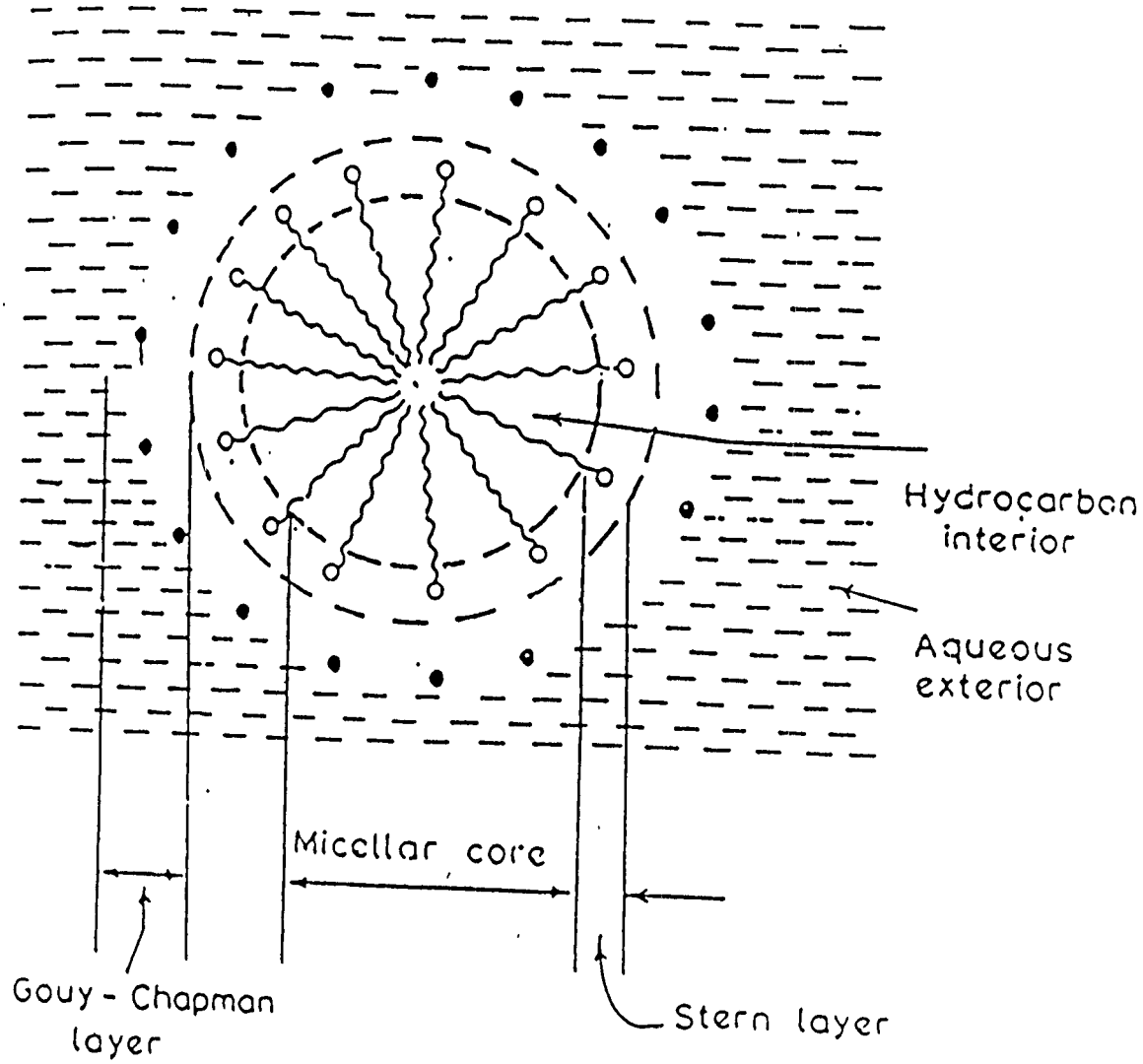



Figure 1.2: Hartley's Model of a Spherical Micelle

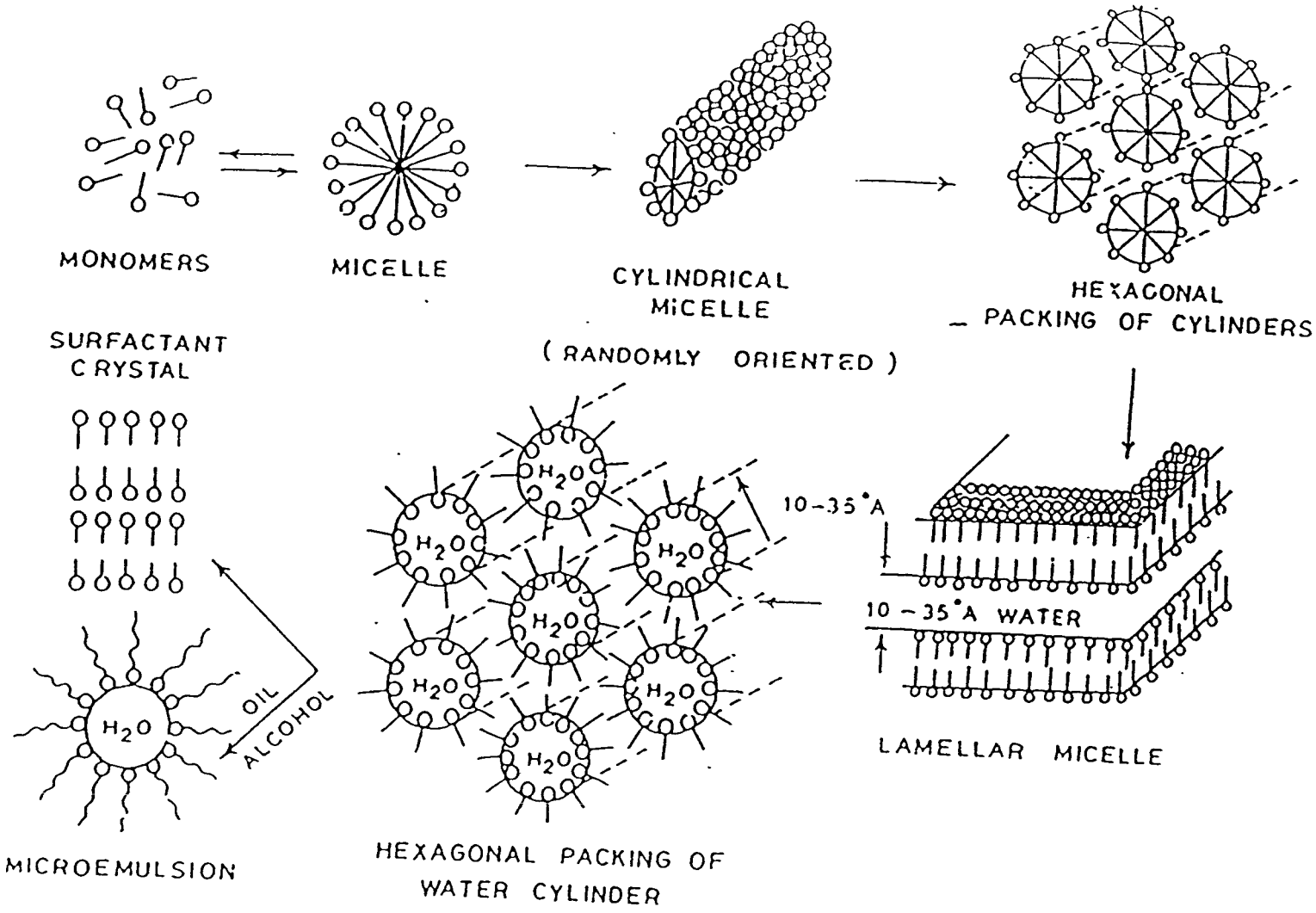



Figure 1.3: A Schematic Illustration for the Formation of Various Structures in Surfactant Solutions upon Increasing the Concentration of Surfactant

A list of some common surfactants is provided in table 1.2.

**Table 1.2 Typical surfactants and their CMC values**

| Surfactant | CMC (M) |
|---|---|
| ***Anionic*** | |
| Sodium dodecyl sulphate (SDS) | $8.1 \times 10^{-3}$ |
| $CH3\ (CH_2)_{11}\ OSO_3^-\ Na^+$ | |
| ***Cationic*** | |
| Cetyl pyridinium chloride (CPC) | $1.2 \times 10^{-4}$ |
| $C_{16}H_{33}\ N^+\ C_5H_5Cl^-$ | |
| Cetyl trimethyl ammonium bromide (CTAB) | $9.0 \times 10^{-4}$ |
| $CH_3\ (CH_2)_{15}\ N^+\ (CH_3)_3\ Br^-$ | |
| ***Non-ionic*** | |
| Poly oxyethylene (6) dodecanol | $9.0 \times 10^{-5}$ |
| $CH_3\ (CH_2)_{11}\ (OCH_2CH_2)_6\ OH$ | |
| Poly oxyethylene p-t - octyl phenol (TX-100) | $1.0 \times 10^{-4}$ |
| $(CH_3)_3\ CCH_2\ (CH_2)_2 - C_6H_4 - (OCH_2CH_2)_{9.5}OH$ | |
| ***Zwitterionic*** | |
| N, N-Dimethyl N- (carboxy methyl) octylammonium salt, | $25 \times 10^{-2}$ |
| $C_8H_{17}N^+\ (CH_3)_2\ CH_2\ COO^-$ | |

## VISUALIZATION

The methods of visualization (detection) used in TLC are of three major types (i) physical (ii) chemical and (iii) enzymatic or biological. Among the physical methods, visualization in UV-light is most common. This methods is highly sensitive, non-destructive, and amenable to the visualization of spots before undertaking quantitative studies. Chemical methods of detection involve the spraying of TLC plates with a suitable reagent, which forms colored compounds with the separated species. *Nanda* and *Devi* have reported an enzymatic method (31) for the detection of heavy metals in fresh water. *Nicolaus*

and *Coronelli* (32) have reported a microbiological method, called bioautography for the detection of antibiotics on TLC plates using triphenyltetrazolium chloride and a micro-organism that is sensitive to the antibiotic in question.

## QUANTITATIVE ANALYSIS

The three main approaches associated with quantitation in TLC are (i) visual estimation, (ii) zone elution and (iii) in- situ densitometry. Amongst these, in-situ densitometry is the preferred technique for quantitative TLC. Substances separated by TLC are quantified by in- situ measurement of absorbed visible or UV-light, or emitted fluorescence upon excitation with UV-light. Absorption of UV- light is measured either on regular layers or on layer with incorporated phosphor.

## ADVANTAGES OF TLC

1. **Simplified sample preparation**

   Irreversible contamination of the stationary phase is not important since this phase is used only once.

2. **Suitable for mass analysis**

   Many samples are simultaneously chromatographed side by side.

3. **Positive identification**

Positive identification is made by co-chromatography of authentic reference substances.

4. **Wider choice of stationary and mobile phases**

   The wider choice of stationary and mobile phases is always possible to achieve particular separation.

5. **Off-line technique**

   Different steps of TLC procedure are carried out independently.

6. **Versatile technique**

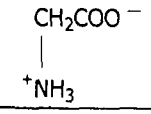
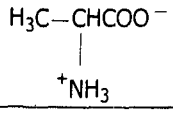
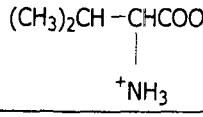
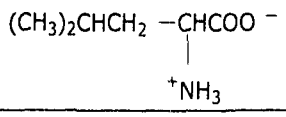
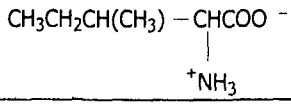
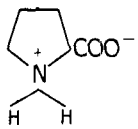
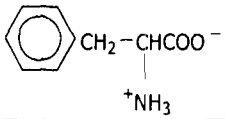
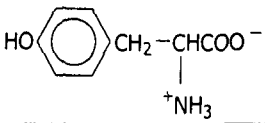
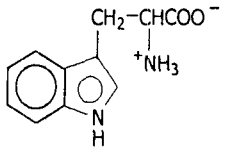
   It may be applied almost for entire spectrum of chemical compounds and it can be performed analytical and preparative scales.

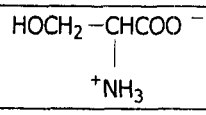
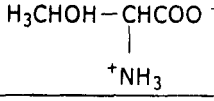
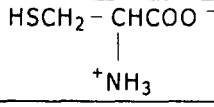
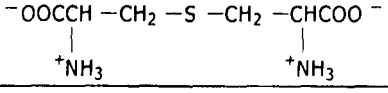
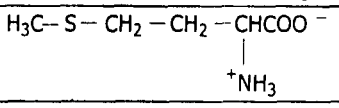
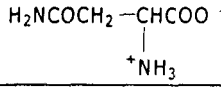
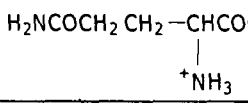
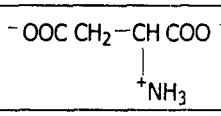
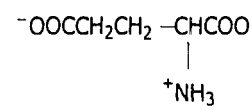
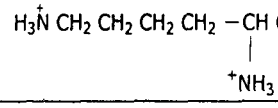
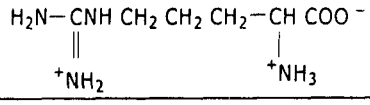
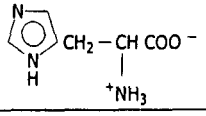
## AMINO ACIDS

These are organic compounds which are sub unit of proteins. Amino acids are bonded together in chains known as peptides. The links between adjacent amino acids is called a peptide bond or link. In 1806 the first amino acid discovered in proteins was asparagine. All amino acids found in proteins have a carboxyl group and an amino group bonded to the same carbon atom. They differ from each other in their side chains, or R groups, which vary in structure, size and electric charge and influence the solubility of amino acids in water.

Amino acids as classified on the basis of the nature of R group are listed in Table 1.3.

**Table: 1.3 Classification of amino acids**

| Amino acids | Structural formula |
|---|---|
| ***(a) Non polar, aliphatic R group*** | |
| Glycine |  |
| Alanine |  |
| Valine |  |
| Leucine |  |
| Isoleucine |  |
| Proline |  |
| ***(b) Aromatic R group*** | |
| Phenylalanine |  |
| Tyrosine |  |
| Tryptophan |  |

| *(c) Polar, Uncharged R group* | |
|---|---|
| Serine |  |
| Threonine |  |
| Cysteine |  |
| Cystine |  |
| Methionine |  |
| Asparagine |  |
| Glutamine |  |
| *(d) Negatively charged R group* | |
| Aspartate |  |
| Glutmate |  |
| *(e) Positively charged R group* | |
| Lysine |  |
| Arginine |  |
| Histidine |  |

## PLAN OF WORK

The present dissertation is aimed to develop optimum and reliable soil thin layer chromatographic systems to understand the

mobility trend of amino acids through static soil bed. Following aspects were investigated.

1. Mobility of amino acids through three types of soil samples.
2. Identification of favourable soil thin layer chromatographic system for selective and separation of amino acids.
3. Exploration of possible use of surfactant, mediated mobile phase system for, separation and detection of amino acids.

## LITERATURE

The research work performed on TLC analysis of organic and inorganic substances had been well documented in the form of several reviews, monographs, books and articles (33-35). CRC Handbook of *chromatography series* started in 1972, under the joint editorship of *G. Zweig* and *J. Sherma,* and continued since 1991 by the latter and the handbook of thin-layer chromatography published in 1992, 1996 and 2003 under the editorship of *B. Fried* and *J. Sherma* have nicely covered the literature of TLC. The work published on TLC of amino acids has during last 15 years presented briefly in Table 1.4.

**Table 1.4: TLC studies of amino acids reported during 1991-2005**

| Title | Analyte | Remarks / Comments | Reference |
|---|---|---|---|
| Localization of amino acids on thin layer chromatograms with acetyl acetone- formaldehyde reagent | Several amino acids | A new reagent, acetyl acetone- formaldehyde is proposed for sensitive detection of separated amino acids on TLC plate under U.V. | 1 |
| Effect of nature of support and impregnating agent on lipophilicity determination for non ionic surfactants by reversed-phase thin layer chromatography. | Fifteen dansylated amino acids derivatives | Salting out and salting in effects in the reversed-phase TLC of dansylated amino acids derivatives using aqueous solution of formic, acetic, propionic and perchloric acids as mobile phase. | 2 |
| Thin layer chromatographic method for the simulataneous determination of physiological aromatic amino acids. | Phenylalanine, tryptophan and tyrosine | Simulataneous determination of amino acids by densitometry on cellulose with sodium sulfate mobile phase. | 3 |


1. M.B. Devani; C.J. Shishoo; S.A. Shah; K.P. Soni; R.S. Shah. *J. Chromatogr.*, 1991, **537**, 494-496
2. T. Cserhati; Z. Illes. *Chromatographia*, 1991, **31**, 152-156.
3. L. Sentier; J. Marchal; J. Boudrant; P. Germain. J. Chromatogr., 1991, **547**, 531-537.

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| Modified ninhydrin spray reagent for the identification of amino acids on thin layer chromatography plates | Several amino acids | Use of D-camphor-10- sulphonic acid-ninhydrin as spray reagent for the identification of amino acids were 0.4-2μg and 0.2-1μg under cold and hot conditions respectively. | 4 |
| Reversed phase planar chromatography of isomers using α-and β-cyclodextrin solutions as eluents. | Tryptophan enantiomers | TLC separation of enantiomers of tryptophans on several type of reversed phase layers with mixed aqueous-organic solutions containing bovine serum albumin. | 5 |
| Determination of D- and L-amino acids in mouse kidney by high-performance liquid chromatography | Amino acids enantiomers | Combination of two dimensional TLC and HPLC for enantiomeric analysis of amino acids of mammalian tissue. | 6 |
| Determination of optical purity of L- amino acids by thin layer densitometry | L- and D- amino acids | Amino acids with D- configuration have lower $R_f$ compared to amino acids with L- configuration on HPTLC precoated chiral plate with Cu salt and optically active amino acids using mixtures of MeCN-$H_2O$ and MeOH or PrOH in different ratio as mobile phase | 7 |
| Adsorption chromatography on cellulose.VII. Chiral separations on cellulose with aqueous solvents. | Enantiomers of tryptophan and phenyl tryptophans | Separation of D- and L- tryptophans and phenyltryptophan by adsorption chromatography with microcrystalline and native cellulose with aqueous solvents. | 8 |


4 S. Laskar; U. Bhattacharya; B. Basak. *Analyst*, 1991, **116**, 625-626.
5 L. Lepri; V. Coas; P.G. Desideri. *J. Planar Chromatogr. Mod. TLC*, 1991, **4**, 338-340.
6 Y. Nagata; K. Yamamoto; T. Shimojo. *J. Chomatogr.*, 1992, **575**, 147-152.
7 M. Suzuki; G. Naitho; S. Takitani. *Iyakuhin Kenkyu*, 1992, **23**, 49-52.
8 M. Lederer. *J. Chromatogr.* 1992, **604**, 55-62.

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| A novel spray reagent for the detection of amino acids by thin –layer chromatography | Several amino acids | A new spray reagent (3,5-dinitrobenzoylchloride) was used for sensitive detection of amino acids (sensitivity, 4-5 µg). The detection was performed under UV light. | 9 |
| Effective solvent systems for separation of PTH-amino acids on polyamide sheet. | PTH-amino acids | Two dimensional TLC, clear separation of all PTH-amino acids in a relatively short time on polyamide sheets. | 10 |
| Chromatographic separation of α-amino acids on antimony (V) phosphate-silica gel "G" plates from some synthetic mixtures and drug samples | Several amino acids | Quantitative separation of amino acids from two drugs (astymin-forte and santevini plus), with antimony (V) phosphate-silica gel "G" and aqueous, non aqueous and mixed solvent systems. | 11 |
| Quantitative determination of L- lysine, L-homoserine, and L-threonine in culture fluids on domestic sorbfil plates by chromatodensitometry | L-lysine, L-homoserine and L-threonine | Quantitative analysis of amino acids in biological fluids. | 12 |
| The possibilities of optical isomer separation by TLC on layers of chitin and its derivatives | L-and D-isomers of amino acids | Separation of optical isomers of amino acids on chitin and transition metals impregnated chitin layers with binary and ternary mobile phases. | 13 |


9 J. C. Kohli;S. K. Grewal. *Natl. Acad. Sci. Lett*, 1992, 15,113-115.

10 G. Mustafa; A. Abbasi; Z. H. Zaidi. *Iran. J. Chem. Chem. Eng.*, 1992, 11, 53-56.

11 K. G. Varshney; A. A. Khan; S. M. Maheshwari; U. Gupta. *Bull. Chem. Soc. Jpn.*, 1992, 65, 2773-2778.

12 Y. V. Degterev; V. F. Panfilov; A. P.Tarasov; B. V. Tyaglov; V. G. Churbanov. *Khim.- Farm. Zh.*, 1992, 26, 121-123.

13 J. K. Rozylo; I. Malinowska. *J. Planar Chromatogr._Mod. TLC*. 1993. 6, 34-37.

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| Reversed phase planar chromatography of dansyl DL amino acids with bovine serum albumin in the mobile phase | Twelve dansyl DL-amino acids | Reversed phase planar chromatographic separation of dansyl DL-amino acids with aqueous organic solutions. | 14 |
| Adsorption chromatography on cellulose IX. Chiral separations with aqueous solvents and liquid-liquid systems | Substituted tryptophan enantiomers | Adsorption chromatography on microcrystalline cellulose with mixed aqueous and organic solvents. | 15 |
| Influence of various salts on the reversed-phase retention of some dansylated amino acids in TLC | Fourteen dansylated amino acids | Reversed-phase TLC and salting out effect on the mobility of amino acids, with aqueous solution of Li, Na, K, Rb, and Cs chlorides. | 16 |
| TLC resolution of DL amino acids on impregnated silica gel plates | Racemic amino acids | Resolution of racemic amino acids on silica gel impregnated with a complex of copper and L- proline with n- butanol- acetonitrile-water (6+2+3), chloroform– methanol- propionic acid (15+6+4) and acetonitrile- methanol- water (92+2+1) as mobile phases. | 17 |


14 L. Lepri; V. Coas; P. G. Desideri; D. Santianni. *Chromatographia,* 1993, 36, 297-301.

15 H. T. K. Xuan; M. Lederer. *J. Chromatogr.*, 1993, 635, 346-348.

16 T. Cerhati; Z. Illes. *Chromatographia,* 1993, 36, 302-306.

17 R. Bhushan; G. P. Reddy; S. Joshi. *J. Planar Chromatogr._ Mod. TLC,* 1994, 7, 126-128.

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| Improved thin- layer chromatographic resolution of PTH-amino acids with some new solvent systems | Eighteen PTH-amino acids | Pyridine- benzene (2.5+20), methanol- $CCl_4$ (1+20) and acetone-$CH_2Cl_2$ (0.3+8), as new mobile phase systems for TLC resolution. | 18 |
| Novel spray reagent for the identification of amino acids on thin-layer chromatography plates | Several amino acids | *p*-Dichlorodicyanobenzoquinone was proposed as a new chromogenic reagent for sensitive detection of amino acids (detection limits,0.1-1μg) on TLC plates | 19 |
| Adsorption chromatography on cellulose. XI.Chiral separations with aqueous solutions of cyclodextrins as eluents | Tryptophan and its fluoro and methyl derivatives | Chiral separations on cellulose with aqueous solution of α-cyclodextrin. | 20 |
| TLC of amino acids on thin silica gel layers impregnated with transition metal ions and their anions | Several amino acids | Improved resolution of amino acids was realized as a result of complexation between amino acids and transition metals on silica gel impregnated with transition metal ions and their anions. | 21 |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19 A. Sinhababu; B. Basak; S. Laskar. *Anal. Proc.*, 1994, **31**, 65-66.

20 H. T. K. Xuan; M. Lederer. *J.Chromatogr.*, 1994, 659, 191-197.

21 R. Bhushan; V. Prasad. *J. Planar Chromatogr._Mod. TLC*, 1994, 7, 480-483

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| Free amino acids in plant extracts.1. Thin layer chromatographic separation and identification of free amino acids | Several amino acids | Identification of free amino acids in extracts of medicinal plants on cellulose. | 22 |
| Kinetic detection of overlapped amino acids in thin-layer chromatography with a direct trilinear decomposition method | Amino acids | Kinetic fluorescence detection of glycine and glutamine after TLC separation. | 23 |
| Simultaneous separation of amino acids, organic acids and nucleosides in clinical chemistry by two-dimensional thin-layer chromatography | Thirty amino acids, twenty nucleotides and related compounds | Two-dimensional TLC for simultaneous separation | 24 |
| A comparative study of HPLC and TLC separation of amino acids using Cu(II) ion | Several amino acids | Utilization of effectiveness of Cu ion in TLC separation of amino acids on silica gel with acetate buffer (0.3 M, pH-6.0)- acetonitrile-n-butanol (12+5+10) mobile phase and the comparison of TLC results with those obtained by RP-HPLC. | 25 |


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23 M. Gui; S. C. Rutan; A. Agbodjan. *Anal. Chem.*, 1995, **67**, 3293-3299.

24 Y. Wu; B. Kuang; F. Long. *Sepu*, 1996, **14**, 259-263.

25 R. Bhushan; I. Ali; S. Sharma. *Biomed. Chromatogr.*, 1996, **10**, 37-39.

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| The separation of D,L-enantiomers of amino acids with thin layer chromatography modified by β-cyclodextrin | Valine, leucine, butyrinc and phenylalanine | Separation of DL-enantiomers of amino acids on silica modified with β-cyclodextrin with urea and dicarboxylic acid containing mobile phase. | 30 |
| Derivative spectroscopy in conjunction with thin layer chromatography applied for determination | Histidine, arginine tryptophan and methionine | Application of TLC in combination of derivative spectroscopy for the determination of amino acids in baby foods on silica gel plates with n-butanol-acetic acid – water (4+1+1) and $C_2H_5OH$- water (70+3) | 31 |
| Separation of optical isomers of amino acids on modified chitin and chitosan layers | Seven DL-mixtures of amino acids | Qualitative analysis of amino acids on chitin, chitosan, Cu impregnated chitin with three-component mobile phases | 32 |
| TLC resolution of enantiomers of amino acids and dansyl derivatives using (1R, 3R, 5R)-2-azobicyclo [3,3,0] octan-3- carboxylic acid as impregnating reagent. | Enantiomers and dansyl derivatives of amino acids | Resolution of enantiomers of amino acids and their dansyl derivatives on impregnated silica gel with mixtures of 0.5M aqueous NaCl and MeCN. | 33 |


30 J. Weng; J. Qiu; Y. Zheng; S. Fujian. *Daxue Xuebao Ziran Kexueban*, 1996, **12**, 31-35.

31 A. Makriyannis; A.A. El Khair; M.M. Ayad; W.S. Hassan; M.M.F. Metwally. *Spectrosc. Lett.* 1997, **30**, 649-660

32 I. Malinowska; J.K. Rozylo. *Biomed. Chromatogr.*, 1997, **11**, 272-275.

33 R. Bhushan; J. Martens; S. Wallbaum; S. Joshi; V. Prasad. *Biomed. Chromatogr.*, 1997, **11**, 286-288.

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| Planar chromatographic direct separation of some aromatic amino acids and aromatic amino alcohols into enantiomers using cyclodextrin mobile phase additives | Racemic aromatic amino acids and aromatic amino alcohols | Qualitative analysis of aromatic amino acids and aromatic amino alcohols on cellulose with highly concentrated solutions of α or β-cyclodextrin | 26 |
| Quantitative thin-layer chromatography of industrial amino acids | Amino acids | Quantitative TLC of industrial amino acids employing video densitometric analytical technique | 27 |
| Modified programmed multiple gradient development (MGD) in the analysis of complex plant extracts | Amino acids derivatives | Separation of derivatives of amino acids on HPTLC silica layers following multiple gradient development technique with different concentrations of ethyl acetate in heptane and chloroform | 28 |
| Study on the limit test of other amino acid in amino acids by thin layer chromatography | Amino acids | A rapid and reproducible TLC method for detection of amino acids (detection limit=0.02 μg) on silica gel 'G' with n-butanol-glacial acetic acid-water (3+1+1) | 29 |


26 M.B. Huang; H.K.Li; G.L. Li; C.T. Yan; L. P. Wang. *J. Chromatogr. A.* 1996, 742, 289-294.

27 I.I. Malakhova; B.V. Tyaglov; E.V. Degterev; V.D. Krasikov; W.G. Degtiar. *J. Planar Chromatogr.-Mod. TLC*, 1996, 9, 375-378.

28 G. Matysik. *Chromatographia* 1996, 43, 39-43.

29 D. Guo; X. Zeng; J. Yang. *Zhongguo Shenghua Yaowu Zazhi*, 1996, 17, 119-121.

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| Thin-layer chromatographic separation of amino acid enantiomers using ligand exchange | Amino acid enantiomers | Qualitative analysis of amino acid enantiomers on silica gel treated with L–arginine and Cu acetate. | 34 |
| Topological indexes for evaluation of the separation of D and L amino acids by TLC | D and L isomers of amino acids | TLC separation of amino acid enantiomers on chiral plates. Distinction between L and D isomers on the basis of proposed topological indexes. | 35 |
| Quantitative analysis of L–lysine L–threonine, L–homoserine and cobalamines *in fermentation* broth | L–lycine, L–threonine, L–homoserine and cobalamine | Quantitative analysis of amino acids on sorbfil TLC plates with mixed-aqueous organic solvents containing $NH_3$. | 36 |
| Quantitative analysis of L–tryptophan in fermentation broth | L–Tryptophan | Quantitative analysis of L–tryptophan on sorbfil TLC plates with propan-2-Ol-25% aqueous $NH_3$ | 37 |


34 Q.Y. Deng; Z. Zhang; J.Y. Su. *Chin. Chem. Lett.*, 1997, 8, 161-164.

35 A. Pyka. *J. Liq. Chromatogr. Relat. Technol.*, 1999, 22, 41-50.

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37 E.V. Degterev; W.G. Degtiar; B.V. Tyaglov; A.P. Tarasov; V.M. Krylov; I.I. Malakhova; V.D. Krasikov. *J. Planar Chromatogr.-Mod. TLC*. 2000, 13, 191-194.

| Title | Analyte | Remarks/Comments | Ref. |
|---|---|---|---|
| Micellar thin layer chromatographic separation and identification of amino acids: Separation of L–proline from some aliphatic and aromatic amino acids. | Aliphatic and aromatic amino acids | Separation of L–proline from other aliphatic and aromatic amino acids by micellar thin-layer chromatography on plain alumina. | 38 |
| Detection, separation and analysis of α-amino acids by means of TLC using 4 dipropylamino- diazabenzene-4'-iso- thiocyanate (DPABITC) | α-Amino acids | DPABITC was synthesized and used to obtain thiohydantoin derivatives of α-amino acids. The colored derivatives were separated by TLC on silica gel using various solvent systems. | 39 |
| Application of microemulsions in thin layer chromatographic analysis of amino acids | Amino acids | TLC analysis of amino acids using microemulsion systems as mobile phase on silica gel. | 40 |
| Resolution of enantiomers of DL-amino acids on silica gel plates impregnated with optically pure (-)-quinine. | Enantiomers of amino acis | TLC resolution of enantiomers from racemic amino acids was achieved on silica gel plates impregnated with optically pure (-)-quinine. The successful solvent systems were butanol-chloroform-acetic acid and Et acetate-$CCl_4$-propionic acid. | 41 |


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| Separation of amino acids on alumina layer developed with with oil-in-water microemulsion | Amino acids | Qualitative separation of aliphatic and aromatic amino acids on plain alumina and $Li^+$, $Na^+$, $NH_4^+$ impregnated alumina with oil-in-water microemulsion. | 42 |
| Effect of mobile phase composition and pH on thin layer chromatographic behaviour of amino acids | Amino acids | The chromatographic behaviour of 24 amino acids was studied on plain silica gel 'G' layers in one component, two-component (butanol-acetic acid) and three-component (acetone-benzene-acetic acid) mobile phases in varying ratios. | 43 |
| Thin layer chromatography of amino acids on titanium tungstate using dimethyl sulfoxide as the mobile phase. | Amino acids | DMSO was used as the mobile phase for TLC of amino acids on titanium tungstate. | 44 |
| Application of a new developer in thin layer chromatographic analysis of amino acids. | Twenty three amino acids | The chromatographic behaviour of amino acids on the silica gel thin layers using CTAB-Bu alc-n-octane-water microemulsion as a developer was studied | 45 |
| Simple methodology for the purification of amino acids | Amino acids | The isolation and separation of amino acids was carried out by silica gel thin layer chromatography using as a eluent a mixture of isopropanol-methanol-$NH_3$ (0:1:1) to (9:1:0.5). | 46 |


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| Two new spray reagents for detection of amino acids on thin-layer plates | Amino acids | The proposed reagents (4-hydroxyacetophenone-isatin and 4- hydroxybenzaldehyde-isatin) were the formation of distinguishable colours with most of the amino acids after final heating. | 47 |
| Detection of proline, arginine, and lysine using iodine-azide reaction in TLC and HPTLC | Proline, arginine and lysine | The application of a modified I-azide procedure for the detection of proline, arginine and lysine is described. The developed plates were sprayed with a mixture of Na azide and starch solution, adjusted to pH 5.5, and exposed to I vapour. | 48 |
| Selective TLC separation of lysine and threonine in pharmaceutical preparations | α - Amino acids | Thin layer chromatography of α-amino acids has been performed on layers prepared from a 1:4 stannic arsenate- cellulose mixture. Lysine and threonine were selectively separated and quantitatively determined from among the mixture of amino acids present in a common available drug. | 49 |
| Mobility behaviour of amino acids through soil TLC | Amino acids | Transportation of amino acids through static flat bed of buffered soil (pH 2.06) as the stationary phase and water in oil microemulsion as the mobile phase. | 50 |


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|---|---|---|---|
| Selective TLC separation of amino acids | Thio amino acids (L-cysteine and L-cystine) | Use of Bis (2-ethylhexyl) sodium sulphosuccinate (AOT) as extractent for selective separation of L-cystine, L-Cysteine, L-methionine | 51 |
| TLC analysis of some organic compounds in some Croation Hypercium Tax | Flavanoids, phenolic acids and amino acids | To identification and determination of amino acids and other compounds | 52 |
| Application of direct dying developing agent of TLC on identification of amino acids | Essential and non essential amino acids | Silica gel G thin layer plates developed with a n-butanol-ninhydrine-pyridine – water solution and then baked be show colour spots of amino acids. | 53 |


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# Chapter-2

## Soil Thin Layer Chromatography of Amino Acids with Water & Surfactant Containing Mobile Phase Systems

# INTRODUCTION

Flat bed or planar chromatography has been considered as one of the simplest analytical techniques for the separation of organic and inorganic substances (1-6). The convenience and cost effectiveness of thin layer chromatography (TLC) have resulted in its wide range of applicability in separation and identification of agro chemicals (7,8), TLC has been successfully employed for rapid analysis of dansylated amino acids by *T. Cserhati et al.* (9). TLC of amino acids using various solvent systems as mobile phase are well documented (10-13). Investigation (14) regarding amino acids metabolism in soil and plants uptake of amino acids in the form of nitrate by plants from soil and amino acids migration in soil by soil TLC has much to offer to the chemists interested in examining the uptake, translocation and degradation of amino acids in the environment.

The present study was taken up with the aim of understanding the mobility pattern of twenty- two amino acids through a static flat phases of soil in contact with surfactant containing aqueous mobile phase systems. The use of surfactants as mobile phase systems have been reported earlier for the separation of amino acids (15,16), inorganic ions (17,18,19) and aromatic amines (20,21). Selected mobile phases in present study are generally encountered with the soil surface and hence the results of transportation of amino acids through

soil bed under the selected experimental conditions will be helpful to formulate the strategy for enhancing the migration of useful nitrogen source (i.e. amino acids) in the soil bed for the healthy growth of plant.

## EXPERIMENTAL

### Appartus

A thin layer chromatography apparatus (Toshniwal, India), 20 x 3.5cm glass plates, 24 x 6cm glass jars and micropipette (0.5-10 μl, Germany) were used.

### Chemicals and Reagents

Sodium dodecyl sulphate, (SDS) and nin hydrine (Merck, India), N-Cetyl N, N trimethyl ammonium bromide, (CTAB) and Cetyl Pyridinium chloride, (CPC) and Poly oxy ethylene p-t-octyl phenol, (Tx-100) (CDH, India) were used.

### Amino Acids Studied

L-Glycine (L-Gly), L-Proline (L-Pro), L-Histidine (L-His), L-Glutamic acid (L-Glu), L-Lysine (L-Lys), L-Tyrosine (L-Tyr), L-Cystine (L-Cys-Cys), L-Cysteine (L-Cys), L-Serine (L-Ser), L-Leucine (L-Leu), L-Arginine (L-Arg), L-Ornithine (L-Orn), L-Alanine (L-Ala), DL-Aspertic acid (DL-Asp), DL-Alanine (DL-Ala), DL-Valine (DL-Val), DL-Phenyl alanine (DL-Phe), DL-Serine (DL-

Ser), DL-Iso leucine, (DL-Ile), DL-Nor Iso leucine (DL-Nor Ile), DL-Tryptophan (DL-Try), DL-2 Amino butyric acid (DL-2 Amb), DL-Methionine (DL-Met), DL-Threonine (DL-Thr),D-Glutamic acid (D-Glu), D-Leucine (D-Leu), D- Alamine (D-Ala).

**Chromatographic System**

**(a) Stationary Phases**

The following stationary phases were used.

| Code | Composition |
|---|---|
| $S_1$ | A.M.U. Fort soil (15 cm depth) |
| $S_2$ | Sikandra Rao Soil (15 cm depth) |
| $S_3$ | Talashpur Soil (15 cm depth) |

**(b) Mobile Phases**

The following mobile phases were used.

| Code | Composition |
|---|---|
| $M_1$ | Double distilled water |
| $M_2$ | Tap water |
| $M_3$ | Saline water |
| $M_4$ | 0.01 M Aqueous solution of SDS |
| $M_5$ | 0.001 M Aqueous solution of SDS |
| $M_6$ | 0.0001 M Aqueous solution of SDS |
| $M_7$ | 0.001 M Aqueous solution of CPC |
| $M_8$ | 0.00001 M Aqueous solution of CPC |
| $M_9$ | 0.001 M Aqueous solution of CTAB |
| $M_{10}$ | 0.00001 M Aqueous solution of CTAB |
| $M_{11}$ | 0.001 M Aqueous solution of Tx-100 |
| $M_{12}$ | 0.00001 M Aqueous solution of Tx-100 |

**Test Solutions**

Test solutions (1%) of amino acids were prepared in double distilled water.

**Soil Samples**

Soil samples ($S_1$ – $S_3$) of natural, uncultivated soil that were collected from the soil surface horizon (15cm depth) at different places in the district of Aligarh & Hathras (India). The sample were dried, grounded and passed through the 100 mesh size sieve to get uniform particle size. The physico chemical properties of soil are given in Table-2.1.

**Detection Reagent**

A 0.3% nin hydrine solution in acetone was used to detect all amino acids.

**Preparation of Soil TLC Plates**

Soil TLC plates were prepared by mixing soil with double distilled water in a 1:1 ratio by w/v. The resultant slurry was mechanically shaken for 5min and then it was coated on to a glass plates with the help of TLC applicator to give a layer of 0.5mm thickness. The selected plates were dried at room temperature.

**Soil TLC Procedure**

The plates were marked with two horizontal lines at distances 2 and 12cm, from the base about 2 μL of test solution was spotted on a thin layer plate with the help of micropipette. The plates were developed in the chosen solvent system by the ascending techniques.

The solvent ascent was fixed to 10cm in all cases. After development, the plates were withdrawn from glass jars and dried at room temperature and sprayed with suitable detectors to locate the positions of analyte as colourful spots. $R_L$ ($R_L$ of leading front) and $R_T$ ($R_T$ of trailing front) values for detected spots were determined and the $R_F$ values were calculated as :

$$R_F = \frac{1}{10}\left[\frac{R_L + R_T}{2}\right].$$

$$\Delta R_F = (R_F)_1 - (R_F)_2$$

where,

$(R_L + R_T)/2$ = The average of leading and trailing distances from the base line.

$(R_F)_1$ =$R_F$ value of one chromatographic system.

$(R_F)_2$ = $R_F$ value of second chromatographic system.

## Results and Discussion

The complete study has been subdivided into the following parts:

**(a) The examination of physico-chemical properties of soil samples**

The important parameters regarding physico-chemical properties of the studied soil samples have been listed in Table 2.1. Mechanical composition affects the water holding capacity, strength and compressibility of soil. pH of soil is an approximate measure of an active fraction of hydrogen ions present in the soil phase.

Hydrogen ions are strongly attracted to the soil surface and they have the power to replace other cations. Electrical conductivity gives the idea about total soluble salts present in soil. Cation exchange capacity refers to the reversible diffusion controlled and stoichiometric process which involves the preference for one ion over another by the adsorbent. Nitrogen is a vitally important plant nutrients. Plants normally content 1-5% nitrogen by weight. It is absorbed by plants as nitrate ($NO_3^-$), nitrite ($NO_2^-$) and ammonium ($NH_4^+$) ions.

**(b) The study of mobility trend of amino acids on different static soil phases**

The chromatography of 22 amino acids was performed on three types of soil ($S_1$-$S_3$) as stationary phases and using 12 mobile phases. The results are summarized in Tables 2.2 - 2.5.

Following thin layer chromatographic systems were used.

(i) **$S_1$ – $S_3$ soils as stationary phases with double distilled water ($M_1$), tap water ($M_2$) and saline water ($M_3$) as eluents**

From the results summarized in Table 2.2 the following mobility trends are noticed.

- Amino acids such as L-Lys, L-Arg and L-Orn show strong interaction with stationary phases ($S_1$-$S_3$) and remain near the point of application ($hR_F$ range, 5 to 25) in all mobile phase systems ($M_1$- $M_3$).
- Amino acids such as L-Gly, L-Pro, L-Glu, L-Cys-Cys, L-Ala, DL- Phe, DL-Try, DL-2Amb, DL-Met, D-Glu, D-Leu and D-Ala show moderate interaction with all stationary phases ($S_1$-$S_3$) and migrated to middle of the TLC plates ($hR_F$ range, 40 - 75).
- Amino acids such as L-Cys, L-Leu, DL-Asp, DL-Val, DL-Ser, DL-Ile, and DL-Thr show strong interaction with all the mobile phases ($M_1$-$M_3$) and weak interaction to all stationary phases ($S_1$-$S_3$). As a result they move to the top of the plate. ($hR_F$ range, 80 - 95).
- Some amino acids (L-Lys, L-Cys, DL-Ala, DL-Val, L-Pro, L- His, L-Glu, L-Tyr, L-Cys-cys, DL-Nor Ile, DL-Try, DL-2Amb, D-Glu and DL-Ser) could not be detected clearly

with certain chromatographic systems. None of the amino acids was detected on $S_3$ when saline water ($M_3$) was used as mobile phase.

- Amino acids such as DL-2Amb, DL-Phe, DL-Ser and D-Leu were found to show occasional tailing ($R_L - R_T \geq 0.3$) and their spots appear as elleptical extension in the direction of solvent flow.

**(ii) $S_1$–$S_3$ soils as stationary phases with aqueous surfactants as eluents**

With surfactant containing mobile phase systems, amino acids show different migrational behaviour as compared with their behaviour in distilled, tap and saline water systems. The results obtained on $S_1$-$S_3$ with aqueous surfactant systems are listed in Tables 2.3-2.5. From these result following conclusions are drawn.

- L-Lys, L-Arg and L-Orn show strong interaction with all stationary phases ($S_1$ - $S_3$), and remain near the point of application ($hR_F$ range, 5 – 25) in all mobile phase systems.
- Amino acids such as L-Gly, L-Pro, L-His, L-Tyr, L-Cys-Cys, L-Cys, D-Glù, D-Leu and D-Ala show moderate interaction with all stationary phases ($S_1$-$S_3$) migrate to middle of the TLC plate, ($hR_F$ range, 40-75). With $M_{11}$-

$M_{12}$, L-Gly migrates to the solvent front on $S_1$-$S_3$ stationary phases showing no interaction with the soil.

- Amino acids such as L-Glu, L-Leu, DL-Asp, DL-Ala, DL-Val, DL-Phe, DL-Ser, DL-Ile, DL-Nor Ile, DL-Tyr, DL-2Amb and DL-Met show strong interaction with the mobile phases ($M_4$-$M_{12}$) and weak interaction to all stationary phases ($S_1$-$S_3$). As a result they move to the top of the soil TLC Plate ($hR_F$ range, 80-95).
- Some amino acid like L-Try, L-Cys-Cys, L-Cys, L-His, L-Pro, DL-Phe, DL-Ser and DL-Thr could not be detected clearly with certain chromatographic systems.
- Amino acids L-Ile, D-Glu, DL-Tyr, L-Gly, D-Leu, D-Ala, DL-Phe, DL-Nor Ile, DL-2Amb, DL-Met, D-Ala, L-Cys-Cys, L-Ser, DL-Asp, DL-Val and DL-Thr, were found to show occasional tailing ($R_L$- $R_T \geq 0.3$) and their spots appear elleptical extension in the direction of solvent flow.

Amino acids such as L-Lys, L-Arg and L-Orn ($hR_F$ range, 10) are separated with other amino acids. That show higher mobility on $S_1$-$S_3$ with all mobile phases ($M_1$-$M_{12}$).

The data presented in Figure 2.1 clearly demonstrate that the mobility trend is changed when Tx-100 is used as impregnant for $S_1$ soil, instead of its use as mobile phase ($M_{11}$) . This figure was

constructed by plotting $\Delta R_F$ values [$R_F$ with Tx-100 in mobile phase and soil as stationary phase minus $R_F$ with Tx-100 as impregnant of soil stationary phase ($S_1$) and distilled water as mobile phase]. it is evident that the mobility is strongly influence by the use of Tx-100 in mobile or stationary phase. The positive value of $\Delta R_F$ show the higher mobility of amino acids when it is used in mobile phase instead of in stationary phase. Conversely, the negative value of $\Delta R_F$ show higher mobility of amino acids with Tx-100 as impregnent of soil instead of its use in mobile phase.

## Table 2.1: Physico- chemical properties of different soils

| Parameters | $S_1$ (AMU Fort) Soil | $S_2$ (Sikandra Rao) Soil | $S_3$ (Talashpur) Soil |
|---|---|---|---|
| (a) Mechanical composition (%) | | | |
| Sand | 62.70 | 61.83 | 71.77 |
| Silt | 24.25 | 24.57 | 18.73 |
| Clay | 13.05 | 13.60 | 9.50 |
| (b) pH (Soil Water 1:5) | 8.4 | 9.2 | 7.9 |
| (c) Electrical conductivity (mMhos/cm) | 0.64 | 1.500 | 0.48 |
| (d) Organic matter (%) | 0.47 | 0.42 | 0.52 |
| (e) Cation exchange capacity (meq./100g soil | 16.3 | 13.8 | 16.1 |
| (f) Exchangeable cations (meq/100g soil) | | | |
| $K^+$ | 0.50 | 0.34 | 0.50 |
| $Na^+$ | 1.00 | 1.96 | 0.49 |
| $Ca^{+2}$ | 3.50 | 5.70 | 7.20 |
| $Mg^{2+}$ | 1.50 | 0.51 | 1.32 |
| (g) Available Nitrogen (ppm) | | | |
| $NH_4^+$ | 52.0 | 33.3 | 50.0 |
| $NO_3^-$ | 40.0 | 17.20 | 35.0 |
| $NO_2^-$ | 6.0 | 25.25 | 29.20 |

**Table 2.2: Mobility ($hR_F$) of amino acids on different stationary phases using double distilled water ($M_1$) and tap water ($M_2$) and saline water ($M_3$) as mobile phases.**

| Amino acids | $S_1$ | | | $S_2$ | | | $S_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | $M_1$ | $M_2$ | $M_3$ | $M_1$ | $M_2$ | $M_3$ | $M_1$ | $M_2$ | $M_3$ |
| L-Gly | 75 | 56 | 70 | 65 | 70 | 65 | 60 | 75 | N.D |
| L-Pro | 47 | 51 | 50 | 45 | 58 | 45 | N.D | N.D | N.D |
| L-His | 32 | 37 | 47 | 37 | 40 | 42 | N.D | N.D | N.D |
| L-Glu | 70 | 57 | 57 | 75 | 55 | 50 | N.D | N.D | N.D |
| L-Lys | 10 | 07 | N.D. | 07 | 07 | N.D. | 05 | 07 | N.D |
| L-Tyr | 27 | 57 | 75 | 27 | 50 | 70 | N.D | N.D | N.D |
| L-Cys-Cys | 45 | 50 | 75 | 60 | 40 | 68 | N.D | N.D | N.D |
| L-Cys | 80 | 88 | 82 | 85 | N.D. | N.D. | N.D | N.D | N.D |
| L-Ser | 85 | 72 | 80 | 85 | 70 | 90 | 75 | 76 | N.D |
| L-Leu | 82 | 87 | 80 | 80 | 80 | 80 | 85 | 82 | N.D |
| L-Arg | 10 | 10 | 20 | 05 | 07 | 15 | 05 | 05 | N.D |
| L-Orn | 10 | 05 | 25 | 05 | 07 | 25 | 15 | 07 | N.D |
| L-Ala | 70 | 60 | 72 | 75 | 58 | 70 | 42 | 50 | N.D |
| DL-Asp | 80 | 85 | 84 | 80 | 90 | 80 | 83 | 85 | N.D |
| DL-Ala | 77 | 80 | 77 | 78 | 90 | 77 | N.D | N.D | N.D |
| DL-Val | 80 | 82 | 85 | 82 | 80 | 85 | N.D | N.D | N.D |
| DL-Phe | 75 | 75 | 70 | 70 | 75(T) | 70 | 67 | 75(T) | N.D |
| DL-Ser | 85 | 80 | 80 | 80 | 82(T) | 80 | N.D | 80(T) | N.D |
| DL-Ile | 82 | 80 | 85 | 80 | 85 | 80 | 83 | 80 | N.D |
| DL-Nor-Ile | 77 | 72 | 65 | 70 | 71 | 65 | N.D | N.D | N.D |
| DL-Try | 72 | 75 | 70 | 70 | 72 | 72(T) | N.D | N.D | N.D |
| DL-2Amb | 70 | 65 | 75(T) | 70 | 70 | 75(T) | N.D | N.D | N.D |
| DL-Met | 75 | 68 | 70 | 72 | 62 | 70 | 75 | 65 | N.D |
| DL-Thr | 80 | 82 | 80 | 85 | 80 | 82 | 85 | 80 | N.D |
| D-Glu | 70 | 60 | 75 | 70 | 60 | 65 | N.D | N.D | N.D |
| D-Leu | 67 | 65 | 65 | 58 | 58 | 55 | 72 | 73(T) | N.D |
| D-Ala | 57 | 68 | 60 | 68 | 55 | 55 | 70 | N.D | N.D |

**ND = Not detected**

**T = Tailed spot** $(R_L - R_T \geq 0.3)$

$hR_F = R_F \times 100$

**Table 2.3: Mobility ($hR_F$) of amino acids on aqueous anionic surfactant (SDS) containing mobile phase as ($M_4$, $M_5$ and $M_6$) on different stationary phases ($S_1 - S_3$)**

| Amino acids | $S_1$ | | | $S_2$ | | | $S_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | $M_4$ | $M_5$ | $M_6$ | $M_4$ | $M_5$ | $M_6$ | $M_4$ | $M_5$ | $M_6$ |
| L-Gly | 72 | 75 | 75 | 45(T) | 41(T) | 43(T) | 67 | 70 | N.D |
| L-Pro | 60 | 70 | 75 | ND | ND | ND | 57 | 55 | N.D |
| L-His | 70 | 67 | 76 | ND | ND | ND | ND | N.D | N.D |
| L-Glu | 95 | 90 | 92 | 90 | 92 | 95 | 94 | 92 | N.D |
| L-Lys | 10 | 08 | 10 | 05 | 05 | 07 | 06 | 05 | N.D |
| L-Tyr | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| L-Cys-Cys | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| L-Cys | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| L-Ser | 85 | 82 | 80 | 80 | 82 | 90 | 82 | 87 | N.D |
| L-Leu | 85 | 87 | 89 | 83 | 80 | 85 | 80 | 90 | N.D |
| L-Arg | 05 | 05 | 07 | 05 | 02 | 03 | 06 | 05 | N.D |
| L-Orn | 05 | 05 | 07 | 05 | 05 | 06 | 08 | 05 | N.D |
| L-Ala | 87 | 85 | 87 | 79 | 82 | 90 | 80 | 82 | N.D |
| DL-Asp | 87 | 80 | 85 | 85 | 80 | 95 | 81 | 80 | N.D |
| DL-Ala | 85 | 82 | 87 | 85 | 80 | 90 | 82 | 90 | N.D |
| DL-Val | 95 | 90 | 97 | 92 | 90 | 85 | 90 | 91 | N.D |
| DL-Phe | 82 | 87 | 88 | ND | ND | ND | 89 | 85 | N.D |
| DL-Ser | 85 | 90 | 92 | ND | ND | ND | 80 | 83 | N.D |
| DL-Ile | 89 | 90 | 91 | 87 | 85 | 90 | 83(T) | 80(T) | N.D |
| DL-Nor-Ile | 85 | 84 | 85 | 83 | 81 | 82 | 85 | 87 | N.D |
| DL-Try | 80 | 81 | 85 | 80 | 87(T) | 85(T) | 90 | 92 | N.D |
| DL-2Amb | 80 | 80 | 82 | 82 | 80 | 85 | 82 | 85 | N.D |
| DL-Met | 80 | 78 | 85 | 80 | 85 | 82 | 85 | 90 | N.D |
| DL-Thr | 82 | 77 | 79 | 83 | 83 | 89 | ND | ND | N.D |
| D-Glu | 67 | 60 | 68 | 70 | 65 | 68 | 75(T) | 50(T) | N.D |
| D-Leu | 62 | 65 | 67 | 75 | 60(T) | 65 | 60(T) | 65 | N.D |
| D-Ala | 70 | 75 | 68 | 72 | 62(T) | 63(T) | 50(T) | 57(T) | N.D |

**ND = Not detected**

**T = Tailed spot** $(R_L - R_T \geq 0.3)$

$hR_F = R_F \times 100$

**Table 2.4: Mobility ($hR_F$) of amino acids on aqueous cationic surfactant (CPC and CTAB) containing different mobile phase as ($M_7$, $M_8$ and $M_9$, $M_{10}$) on different stationary phases ($S_1$ – $S_3$)**

| Amino acids | $S_1$ | | | | $S_2$ | | | | $S_3$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $M_7$ | $M_8$ | $M_9$ | $M_{10}$ | $M_7$ | $M_8$ | $M_9$ | $M_{10}$ | $M_7$ | $M_8$ | $M_9$ | $M_{10}$ |
| L-Gly | 52 | 61 | 65 | 67 | 37 | 40 | 57 | 52 | 68 | 62 | 70 | 71 |
| L-Pro | 42 | 70 | 50 | 52 | 47 | 45 | 45 | 48 | 50 | 40 | 42 | 50 |
| L-His | 63 | 60 | 47 | 62 | 66 | 65 | 38 | 40 | 45 | 50 | 50 | 60 |
| L-Glu | 91 | 90 | 80 | 87 | 85 | 80 | 84 | 81 | 87 | 95 | 85 | 86 |
| L-Lys | 05 | 10 | 08 | 07 | 05 | 05 | 10 | 05 | 05 | 06 | 07 | 05 |
| L-Tyr | 55(T) | 60(T) | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| L-Cys-Cys | 62 | 60 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| L-Cys | 70 | 60 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| L-Ser | 95 | 77 | 78 | 77 | 80 | 85 | 88 | 80 | 96 | 87 | 85 | 84 |
| L-Leu | 96 | 88 | 85 | 87 | 82 | 85 | 80 | 82 | 97 | 95 | 90 | 81 |
| L-Arg | 05 | 07 | 06 | 05 | 05 | 02 | 05 | 05 | 03 | 02 | 06 | 07 |
| L-Orn | 06 | 06 | 05 | 06 | 05 | 07 | 06 | 05 | 04 | 05 | 06 | 07 |
| L-Ala | 80 | 85 | 87 | 85 | 82 | 85 | 87 | 80 | 77 | 86 | 80 | 78 |
| DL-Asp | 85 | 88 | 85 | 87 | 80 | 83 | 84 | 85 | 85 | 88 | 82 | 81 |
| DL-Ala | 80 | 81 | 81 | 83 | 84 | 82 | 87 | 82 | 80 | 82 | 85 | 80 |
| DL-Val | 87 | 86 | 84 | 90 | 82 | 85 | 83 | 86 | 84 | 82 | 82 | 80 |
| DL-Phe | 84 | 80 | 81 | 87 | 82 | 82 | 85 | 82 | 82 | 80(T) | 85(T) | 86(T) |
| DL-Ser | 83 | 80 | 90 | 82 | 80 | 82 | 80 | 82 | 80 | 85 | 80 | 81 |
| DL-Ile | 85 | 85 | 85 | 85 | 85 | 82 | 80 | 85 | 85 | 80 | 85 | 88 |
| DL-Nor-Ile | 82 | 86 | 81 | 87 | 84 | 90 | 85 | 86 | 82 | 82 | 82 | 92(T) |
| DL-Try | 92 | 93 | 80 | 85 | 86 | 92 | 81 | 85 | 81 | 82 | 84 | 93(T) |
| DL-2Amb | 88 | 90 | 92 | 90 | 85 | 90 | 89 | 87 | 88 | 85 | 80 | 92(T) |
| DL-Met | 85 | 80 | 85 | 89 | 95 | 82 | 79 | 77 | 85 | 85 | 83 | 90(T) |
| DL-Thr | 78 | 82 | 80 | 85 | 85 | 77 | 76 | 79 | 80 | 82 | 79 | 82 |
| D-Glu | 65 | 65 | 60 | 69 | 62 | 65 | 66 | 52 | 67 | 65 | 71 | 75 |
| D-Leu | 63 | 61 | 67 | 60 | 65 | 69 | 60 | 70 | 72(T) | 75(T) | 51(T) | 59(T) |
| D-Ala | 70 | 67 | 63 | 65 | 68 | 58 | 65(T) | 60(T) | 55(T) | 57(T) | 79(T) | 68(T) |

**ND = Not detected**

**T = Tailed spot** $(R_L - R_T \geq 0.3)$

$hR_F = R_F \times 100$

**Table 2.5: Mobility ($hR_F$) of amino acids on aqueous non ionic surfactant (Tx – 100) containing mobile phase, ($M_{11}$, $M_{12}$) on different soil samples as stationary phases.**

| Amino acids | $S_1$ | | $S_2$ | | $S_3$ | |
|---|---|---|---|---|---|---|
| | $M_{11}$ | $M_{12}$ | $M_{11}$ | $M_{12}$ | $M_{11}$ | $M_{12}$ |
| L-Gly | 80 | 85 | 95 | 87 | 80 | 85 |
| L-Pro | 55 | 52 | 70 | 72 | ND | ND |
| L-His | 70 | 75 | 72 | 75 | 55 | 60 |
| L-Glu | 87 | 85 | 85 | 85 | 87 | 90 |
| L-Lys | 07 | 05 | 08 | 07 | 07 | 05 |
| L-Tyr | 75 | 70 | 72 | 70 | 70 | 77 |
| L-Cys-Cys | 65 | 60 | 65 | 65 | 80 (T) | 85 (T) |
| L-Cys | 55 | 52 | 50 | 55 | 45 | 48 |
| L-Ser | 87 | 80 | 80 | 80 | 52 (T) | 60 (T) |
| L-Leu | 95 | 90 | 90 | 90 | 92 | 90 |
| L-Arg | 06 | 05 | 05 | 05 | 06 | 06 |
| L-Orn | 07 | 07 | 08 | 08 | 06 | 07 |
| L-Ala | 83 | 80 | 85 | 70 | 83 | 85 |
| DL-Asp | 92 | 90 | 85 | 80 | 92 (T) | 95 (T) |
| DL-Ala | 85 | 80 | 85 | 80 | 85 | 90 |
| DL-Val | 85 | 83 | 82 | 80 | 90 (T) | 95 (T) |
| DL-Phe | 92 | 90 | 90 | 92 | 90 | 95 |
| DL-Ser | 95 | 94 | 90 | 90 | 91 | 95 |
| DL-Ile | 94 | 90 | 90 | 92 | 87 | 90 |
| DL-Nor-Ile | 88 | 84 | 85 | 80 | 90 | 95 |
| DL-Try | 90 | 88 | 92 | 90 | 95 | 97 |
| DL-2Amb | 88 | 85 | 85 | 85 | 88 | 90 |
| DL-Met | 85 | 80 | 82 | 80 | 80 (T) | 80 (T) |
| DL-Thr | 79 | 78 | 80 | 85 | 79 (T) | 78 (T) |
| D-Glu | 65 | 69 | 68 | 75 | 70 | 75 |
| D-Leu | 65 | 70 | 60 | 70 | 72 | 68 |
| D-Ala | 72 | 75 | 62 | 72 | 65 | 70 |

**ND = Not detected**

**T = Tailed spot** $(R_L - R_T \geq 0.3)$

$hR_F = R_F \times 100$

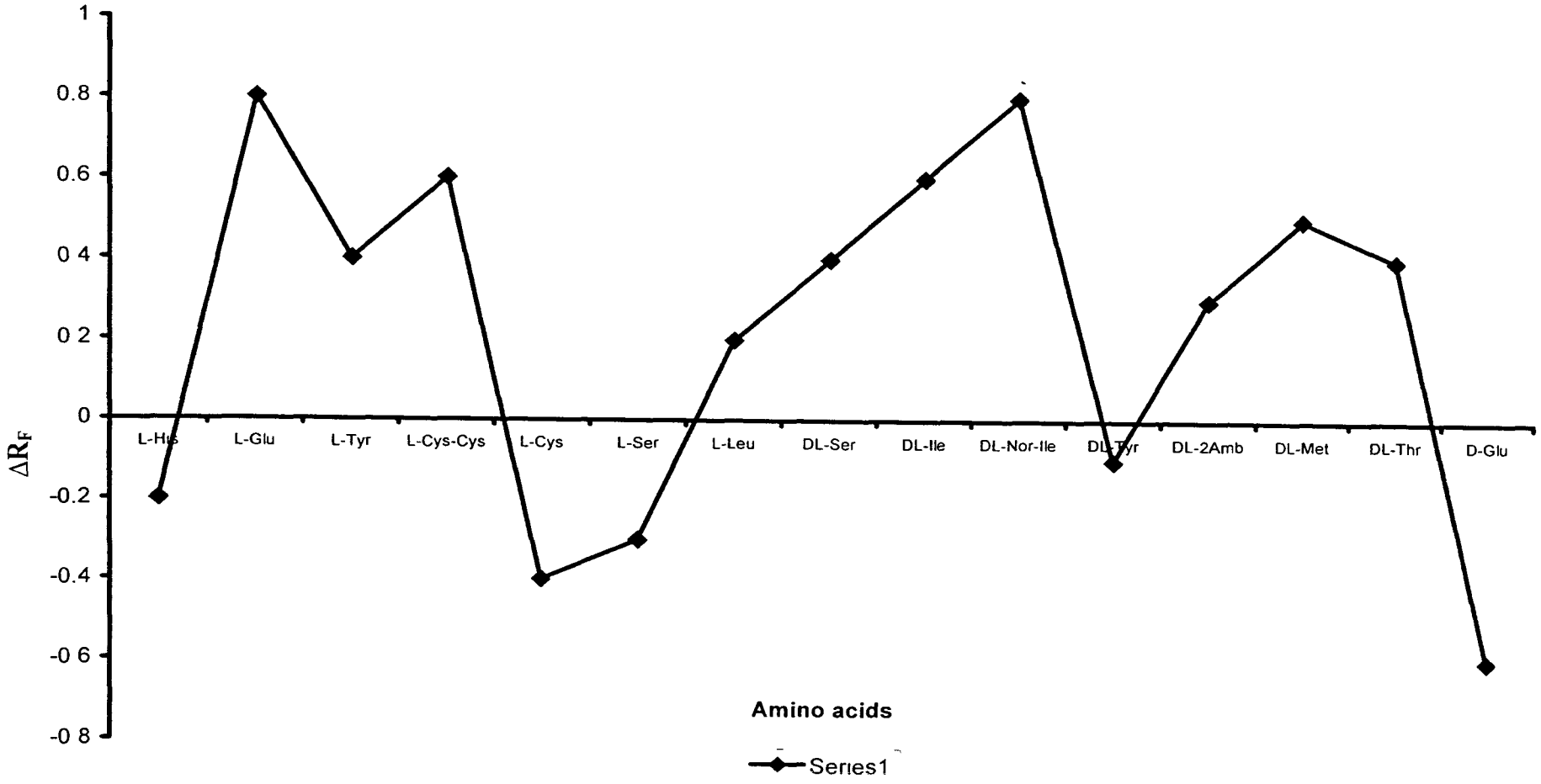


**Figure 2.1:** **Plot of $\Delta R_F$ ($\Delta R_F = R_F$ with Tx-100 in mobile phase and as stationary phase minus $R_F$ with Tx-100 as impregnant of soil stationary phases and distilled water as mobile phase vs amino acids chromotographed on ($S_1$, soil)**

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# Conclusion

# CONCLUSION

This thesis deal with the physico chemical analysis of soil and the utility of soil static bed as an analytical tool to study the mobility pattern of amino acids. The obtained results may be useful to understand the transportation of amino acids through double distilled, tap and saline waters and aqueous surfactants solution. The poorer results were obtained on Talashpur Soil ($S_3$) whereas soil samples collected from AMU fort (soil, $S_1$) and Sikandra Rao (soil, $S_2$) gave good results in terms of clearer detection, spot compactness and separation efficiency. The transportation characteristics of amino acids depends on the composition (or nature) of both stationary (i.e. soil) and mobile (i.e. water and aqueous surfactant) phases.